Hox genes play a critical role in the patterning of the axial skeleton. This has been clearly demonstrated in mice mutant for the entire *Hox10* or *Hox11* paralogous group. *Hox10* triple mutants demonstrate an anterior homeotic transformation of all lumbar vertebrae toward a thoracic fate with rib processes protruding from each vertebral segment through the lumbar and sacral regions. *Hox11* triple mutants display normal development of the thoracic and lumbar region; however, no sacral vertebrae are formed. The vertebral elements in the sacral region are transformed to a lumbar fate. Although genetic analyses have provided important insight regarding *Hox* gene patterning of the axial skeleton, the molecular mechanisms involved in this process are not understood. In order to elucidate the genes and pathways that are regulated by Hox in axial patterning, I have performed microarray analysis on isolated sclerotomal cells, the precursors of vertebrae, from wild type and *Hox11* triple paralogous mutants at several developmental stages to identify genes that are differentially expressed in these animals. This analysis has uncovered differential expression of several genes, including several BMP pathway members. *In situ* hybridization analyses have shown that *Bmp2* expression is significantly reduced in the developing sacral region of *Hox11* mutants. Together, our data suggest that regional *Hox* expression might control localized expression of *Bmps* during morphogenesis of the axial skeleton.

doi:10.1016/j.ydbio.2009.05.494

Program/Abstract # 467
Hox genes control the timing of somite precursor cells ingression during gastrulation in the chicken embryo
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A striking characteristic feature of the spine is the subdivision of groups of vertebrae into anatomical domains such as the cervical, thoracic, lumbar, sacral and caudal regions. This axial regionalization is controlled by a set of transcription factors called Hox genes. These genes are arranged along chromosomal domains, which are linearly deployed during embryonic development – a property termed colinearity. This striking genomic organization is translated into the colinear Hox expression domains during gastrulation. Recently, it has been shown that the genes from the *Hoxb* cluster are activated in the somite precursors of the epiblast in a temporal sequence that reflects their colinear arrangement and subsequently controls the progressive ingression of somite precursors into the nascent paraxial mesoderm (Iimura et al., 2006). Because the *Hoxa*, *c* and *d* clusters are expressed also in the epiblast during gastrulation, we explored the hypothesis of a conserved role of Hox paralogs during this process by overexpressing various Hox genes from the four clusters using the successive electroporation technique in the chicken embryo. We show that all of the paralogs we tested control the timing of epiblast cells ingression into the primitive streak in a colinear fashion. In parallel, we are currently using a microarray-based approach to identify the Hox target genes responsible for the progressive ingression of the somite precursors.

doi:10.1016/j.ydbio.2009.05.495

Program/Abstract # 468
Role of Hox11 genes in anteroposterior patterning of nephrogenic mesenchyme
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Hox genes are critically important for anteroposterior (AP) patterning in a wide variety of organisms. Specific spatial and temporal expression of *Hox* genes along the AP body axis is necessary for proper embryonic development. In the mammalian kidney, it has been shown that *Hox11* paralogous genes are essential for ureteric bud induction, one of the first steps in kidney organogenesis. Further work has demonstrated that Hox11 proteins directly regulate *Six2* and *Gdnf* to control these early processes. Embryos in which five of the possible six alleles are mutated do not exhibit loss of induction, but demonstrate severe AP patterning defects in the nephrogenic mesenchyme. In five allele mutants, the mesonephros persists at later developmental stages and the mesonephric mesenchyme does not separate from the metanephric mesenchyme. These animals die perinatally due to hydronephrosis at the ureteric pelvic junction. Here, we begin to analyze the structural and molecular phenotype of the nephrogenic mesenchyme in these mutants. We believe that these studies will lead to an understanding of how *Hox* genes pattern the nephrogenic mesenchyme along the AP axis.

doi:10.1016/j.ydbio.2009.05.496

Program/Abstract # 469
Functional relevance of Hox-specified positional identities in adult vasculature
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Hoxa3 and *Hoxc11* are expressed in vascular smooth muscle cells (VSMCs) and endothelial cells (ECs) in regionally restricted patterns that closely resemble their respective embryonic expression domains (Pruett et al., 2008). To investigate whether this regionalized expression plays a role in determining the physiological diversification of vessel segments we explored the functional relevance of *Hoxc11* in VSMCs both in vitro and in vivo. Primary cultures of VSMCs established from explanted hindlimb vessel segments of *Hoxc11* reporter mice revealed persistent reporter transgene expression in distinct VSMC subpopulations facilitating phenotypic characterization of *Hoxc11*-positive versus -negative VSMCs. In vitro wound healing and serum-response assays provide evidence that *Hoxc11* expression promotes differentiation towards a contractile SMC phenotype. These results were supported by subsequent functional assays involving *Hoxc11*-transfected mouse vascular cells (MOVAS). These in vitro functional data suggest an important role for *Hoxc11* in the regulation of the phenotypic properties of VSMCs.To study the functional relevance of *Hoxc11* expression in vivo we adopted an innovative murine, doxycycline (Dox)-inducible transgene system, which results in the systemic over-expression of *Hoxc11* in VSMCs using VSMC-specific control elements of the Transgelin (SM22-alphaA) promoter. Together these in vitro and in vitro analyses demonstrate a significant role for Hox code-specified positional identities in the vascular network.

doi:10.1016/j.ydbio.2009.05.497

Program/Abstract # 470
Identification and characterization of Six1 enhancers
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Six1, one of the members of Six homeobox family genes, is expressed in sensory organs and ganglia such as olfactory epithelium,

inner ear, trigeminal and distal cranial ganglia, as well as in somites and nephrogenous mesenchyme in mice. Cranial sensory organs and ganglia are derived from thickened ectoderm termed cranial placodes, which are derived from pre-placodal region (PPR), a continuous ectodermal region surrounding the neural plate. *Six1* is also known as a marker for the PPR and placodes. Analyses of *Six1*$^{-/-}$ mice revealed the essential roles of *Six1* in the development and morphogenesis of the organs where *Six1* is expressed. To identify the enhancers responsible for the expression of *Six1* during embryogenesis, we compared genome sequences around *Six1* loci among vertebrates and found out 16 conserved non-coding sequences (CNSs). The identified CNSs were hooked onto a minimal promoter with EGFP reporter and electorporated into chick embryos to monitor enhancer activities. We identified eight independent enhancers that showed specific expression similar to the endogenous *Six1* expression domains. The enhancer activities were confirmed in mice harboring the CNS upstream of minimal promoter with lacZ reporter. Elements for the CNS that showed expression in the PPR were analyzed by mutagenesis, and homodomain protein binding sites in the CNS were identified as essential for the enhancer activity in the PPR. The involvement of Dlx5 and Msx1 was suggested by overexpression and RNAi experiments in chick embryo. The evolution of *Six1* enhancers will be also discussed.

doi:10.1016/j.ydbio.2009.05.498

Program/Abstract # 471
Dual functions of the miR-10 locus miRNAs in refinement of Hox gene expression
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Controlled regulation of gene expression is essential to proper development. This control can be imposed at nearly every step between initiation of transcription and the eventual degradation of a protein. The discovery of miRNAs demonstrated pervasive post-transcriptional regulation by an ever expanding group of small RNAs which can be expressed in temporally and spatially restricted patterns similar to protein coding genes. The mir-10 locus, which resides in between the Hox4 and Hox5 orthologs in most bilaterian animals, encodes two functional miRNAs miR-10 and miR-10*, which have highly conserved complementary sequences in the 3′UTRs of insect Scr and Abd-Borthologs respectively. These miRNAs and Hox genes in *Drosophila* are expressed in highly complementary and largely non-overlapping domains, suggesting that while the mir-10 miRNAs do not contribute to the gross pattern of Hox gene expression, they are responsible for maintaining precise and developmentally robust expression patterns.

doi:10.1016/j.ydbio.2009.05.499

Program/Abstract # 472
Segmental origin and Hox dependence of neural crest-derived otic ganglion
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Classic studies in chick-quail chimeric embryos show that parasympathetic motor ganglia arise from preotic and postotic segments of the developing hindbrain. Although this observation provides a broad view of the segmental origin of parasympathetic ganglia, it suggests that individual ganglion may arise from rhombomere (r)-specific neural crest cells (NCCs). In turn, the NCCs may be controlled by the determinants of rhombomere identity, the Hox genes. To address these issues, we performed genetic fate maps of Hox gene-expressing Cre and ROSA-EYFP lineage reporter mouse lines to label NCCs originating from specific rhombomeres along the rostrocaudal axis. The identification of individual parasympathetic motor ganglion derived from specific Hox lineage reporter lines was subsequently matched with corresponding Hox knockout mice to determine its dependency on Hox gene function. Using a Hoxa3 lineage reporter line, we show that the otic ganglion, whose fate had not been previously mapped, originates from r6. We found that r6 NCC-derived otic ganglion is independent of Hoxa3 and Hoxb3, the Hox3 paralogous (P) genes known to synergize in r6, but instead require the Hox1P genes, Hoxa1 and Hoxb1. In the absence of the Hox1P genes, the otic ganglion is almost eliminated. This defect is associated with increased apoptosis and loss of dorsal rhombomere identity, as indicated by the absence of Kreissler/Mabf protein expression, which normally labels r5 and r6. These findings suggest that individual parasympathetic motor ganglion originates exclusively from a single rhombomere and depends on the combined function of Hox paralogous genes.

doi:10.1016/j.ydbio.2009.05.500

Program/Abstract # 473
Pax7-SUMOylation and neural crest development
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The paired-box transcription factor Pax7 is expressed in the dorsal neural tube, neural crest cells (NCCs) and somite tissues during vertebrate development. Pax7 is also expressed in muscle satellite cells during adulthood and has been shown to be critical for muscle homeostasis. Recently Pax7 was shown to be a required early marker of NCC precursors. Despite its apparent relevance, little is known about how Pax7 operates, whether it plays a similar role in all these cells or if it provides specific traits to all or any of them. In an effort to further our understanding of the distinct role(s) played by Pax7 during NCC development, we performed a yeast two hybrid screen and identified the SUMOylase enzyme Ubc9 as a novel Pax7 partner. We have verified the interaction of Pax7 with Ubc9 through GST pull down assays and present *in situ* hybridization and immunostaining expression data suggesting their co-expression. Furthermore, *in vitro* and *in vivo* experiments demonstrate the SUMOylation of Pax7, and suggest an early role during neural crest development. Additionally this study unveils an unexpected enrichment of SUMOylation machinery in the neural plate border where prospective NCCs reside. We further provide evidence of the requirement of the SUMO pathway, during early neural crest development.

doi:10.1016/j.ydbio.2009.05.501

Program/Abstract # 474
The role of zebrafish zic genes in neural crest development
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Zic genes encode a conserved family of zinc finger transcription factors. We are focused on zic2a and zic5, which are closely linked and similarly expressed at the neural plate border and throughout the dorsal neural tube during neurula stages. Studies in mouse and *Xenopus* have identified zic2a and zic5 as important regulators of neural crest (NC) induction and perhaps migration, but have not explored these roles in detail. We have observed a severe reduction in jaw cartilage formation in embryos injected with morpholinos that